SUMMARY OF THE INVENTION

[0015] The inventors have identified the structure of the bacterial amyloid secretion channel CsgG. The CsgG channel is a trans-membrane oligomeric protein that forms a channel with a minimum diameter of approximately 0.9 nm. The structure of the CsgG nanopore renders it suitable for use in protein sensing applications, in particular in nucleic acid sequencing. Modified versions of the CsgG polypeptide may serve to further enhance the suitability of the channel for such particular applications.

[0016] The CsgG pore offers an advantage over existing protein pores such as ClyA or alpha-hemolysin in that the structure is favourable for DNA sequencing applications. The CsgG pore has a more favourable aspect ratio comprising a shorter trans-membrane channel than ClyA. The CsgG pore also has a wider channel opening compared to the alpha-hemolysin pore. This can facilitate the attachment of enzymes for certain applications, for example nucleic acid sequencing applications. In these embodiments, it can also minimize the length of the nucleic acid strand section positioned between the enzyme and the reading head (defined as the narrowest pore section) leading to an improved read-out signal. The narrow inner constriction of the channel of the CsgG pore also facilitates the translocation of single stranded DNA in embodiments of the invention involving nucleic acid sequencing. The constriction is composed of two annular rings formed by juxtaposition of tyrosine residues at position 51 (Tyr 51) in the adjacent protein monomers, and also the phenylalanine and asparagine residues at positions 56 and 55 respectively (Phe 56 and Asn 55). The dimensions of the constriction can be modified. ClyA has a much wider inner constriction which allows the passage of double stranded DNA which is currently not used for sequencing. The alpha-hemolysin pore has one 1.3 nm-wide inner constriction but also a 2 nm-wide beta barrel which features additional reading heads.

[0017] In a first aspect, the invention relates to a method for molecular sensing comprising:

[0018] a) providing a CsgG biological pore formed of at least one CsgG monomer within an insulating layer;

[0019] b) applying an electrical potential across the insulating layer thereby establishing flow of electrical current through the biological pore;

[0020] c) contacting the CsgG biological pore with a test substrate; and

[0021] d) measuring the electrical current flow through the biological pore.

[0022] Typically, the insulating layer is a membrane, such as a lipid bilayer. In an embodiment, the electrical current through the pore is carried by a flow of soluble ions from a first side of the insulating layer to the second side of the insulating layer.

[0023] In an embodiment of the invention, the molecular sensing is analyte detection. In a specific embodiment, the method for analyte detection comprises after step (d) the further step of determining the presence of the test substrate by a reduction in electrical current through the biological pore compared to the electrical current through the biological pore when the test substrate is absent.

[0024] In an alternative embodiment of the invention, the molecular sensing is nucleic acid sequencing. Typically, the type of nucleic acid sequenced by said method is DNA or RNA. In specific embodiments of the invention, the CsgG biological pore is adapted to accommodate additional accessory proteins. Typically, the additional accessory proteins are nucleic acid-processing enzymes selected from the group consisting of: DNA or RNA polymerases; isomerases; topoisomerases; gyrases; telomerases; exonucleases; and helicases.

[0025] In embodiments of the invention, the CsgG biological pore is a modified CsgG pore, wherein the modified CsgG pore has at least one modification to the monomeric wild-type *E-coli* CsgG polypeptide sequence in at least one of the CsgG monomers forming the CsgG pore. Typically, the same modification is made to all the CsgG monomers forming the CsgG pore. In specific embodiments of the invention, the modified CsgG monomer has a polypeptide sequence from positions 38 to 63 according to SEQ ID NOs 4 to 388.

[0026] In a second aspect, the invention relates to modified CsgG biological pore comprising at least one CsgG monomer, wherein the modified CsgG biological pore has no more than one channel constriction with a diameter in the range from 0.5 nm to 1.5 nm. Typically, the modification is between positions 38 to 63 of the CsgG monomeric polypeptide sequence. Suitably, the modification is at a position selected from: Tyr51; Asn55; and Phe 56. In specific embodiments, the modification is at position Tyr 51, or at both of positions Asn55 and Phe56.

[0027] In embodiments of the invention, the modification to the CsgG monomer is selected from the group consisting of substitution of the naturally occurring amino acid; deletion of the naturally occurring amino acid; and modification of the naturally-occurring amino acid side chain. Suitably, the modification reduces or removes the steric encumbrance of the unmodified amino acid. In specific embodiments, at least one CsgG monomer of the pore has a polypeptide sequence from positions 38 to 63 according to SEQ ID NOs 4 to 388.

[0028] In a third aspect, the invention relates to the isolated polypeptide encoding the at least one CsgG monomer of the modified CsgG biological pore of the second aspect of the invention.

[0029] In a fourth aspect, the invention relates to isolated nucleic acids encoding the isolated polypeptides of the third aspect of the invention.

[0030] In a fifth aspect, the invention relates to a biosensor comprising:

[0031] a) An insulating layer;

[0032] b) A CsgG biological pore within the insulating layer; and

[0033] c) Apparatus for measuring an electrical current through the biological pore.

[0034] In specific embodiments, the CsgG biological pore in the biosensor is a modified CsgG biological pore according to the second aspect of the invention.

[0035] In a sixth aspect, the invention relates to the use of a CsgG biological pore for biological sensing applications, wherein the biological sensing application is analyte detection or nucleic acid sequencing. In an embodiment of the sixth aspect of the invention, the nucleic acid sequencing is DNA sequencing or RNA sequencing.

[0036] The inventors have surprisingly demonstrated that CsgG and novel mutants thereof may be used to characterise analytes, such as polynucleotides. The invention concerns mutant CsgG monomers in which one or more modifications have been made to improve the ability of the monomer to interact with an analyte, such as a polynucleotide. The